Bioavailability of iodine in the UK-Peak District environment and its human bioaccessibility: an assessment of the causes of historical goitre in this area

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Abstract

Iodine is an essential micronutrient for human health. Its deficiency causes a number of functional and developmental abnormalities such as goitre. The limestone region of Derbyshire, UK was goitre-endemic until it declined from the 1930's and the reason for this has escaped a conclusive explanation. The present study investigates the cause(s) of goitre in the UK-Peak District area through an assessment of iodine in terms of its environmental mobility, bioavailability, uptake into the food chain, and human bioaccessibility. The goitre-endemic limestone area is compared with the background millstone grit area of the UK-Peak District. The findings of this study show that 'total' environmental iodine is not linked to goitre in the limestone area, but the governing factors include iodine mobility, bioavailability and bioaccessibility. Compared with the millstone grit area, higher soil pH and calcium content of the limestone area restrict iodine mobility in this area, also soil organic carbon in the limestone area is influential in binding the iodine to the soil. Higher calcium content in the limestone area is an important factor in terms of strongly fixing the iodine to the soil. Higher iodine bioaccessibility in the millstone grit than the limestone area suggests that its oral bioaccessibility is restricted in the limestone area. Iodine taken up by plant roots is transported freely into the aerial plant parts in the millstone grit area unlike the limestone area, thus providing higher iodine into the human food chain in the millstone grit area through grazing animals unlike the goitre-prevalent limestone area.

Keywords

Bioaccessibility, bioavailability, docks, iodine, Peak District

1. Introduction

Iodine concentrations in the lithosphere have been reported to be generally low, with average concentrations being 0.25 mg/kg (Fuge, 2007). The ocean is the primary source of terrestrial iodine, sea water contains approximately 60 mg/L iodine, and volatilisation from the ocean and precipitation of water vapour is the origin of most of the iodine entering into the food chain (Fuge and Johnson, 1986; Fuge, 2005; Fuge 2007).

Iodine retention in soils and its availability to plants is reported to differ considerably between soil types. Organic-rich soils are frequently enriched in iodine, whereas sandy soils and waterlogged soils are generally depleted in iodine (Fuge, 2007). Iodine can be cycled from the soils into ground and surface waters by leaching, and passed into the food chain by uptake into crops (Whitehead, 1984; Dai et al. 2004). The reactions of iodine within the soil (such as retention by solid phase components, oxidation-reduction, and volatilisation) influence the availability of iodine to plants (Whitehead, 1984; Hu et al. 2005). Soil organic matter, for example, appears to restrict the availability, probably due to the sorption of iodine (Whitehead, 1975; Fuge, 2005). Iodine taken up from soil is mainly retained in the roots of plants, and transport to the shoots may increase with an increase of soil iodine (Whitehead, 1973; Zhu et al. *2003*; Weng et al. 2008). Markert (1992) reported that the iodine content of a hypothetical 'Reference Plant' (excluding element accumulator or rejector plants) is 3 mg/kg.

Although iodine is taken up by plants, its essentiality to plants has not been established (Whitehead, 1984; Fuge and Johnson, 1986; Mackowiak and Grossl, 1999; Kabata-Pendias and Pendias, 2001; Dai et al. 2004). It is believed that iodine does not have any metabolic activity in the plant, so an iodine deficient environment does not result in poor plant growth (Fuge and Johnson, 1986).

Iodine is an essential micronutrient for human health (Kelly and Snedden, 1960; Fraga, 2005). Its deficiency causes a number of functional and developmental

abnormalities collectively grouped as 'Iodine Deficiency Disorders (IDD)', goitre being the most visible manifestation of IDD (WHO, 2004) as an enlargement of the thyroid gland in the front of the neck. The dietary iodine requirement for humans is 150 μg/day (ICCIDD, 2012).

Plant and plant-based products contribute to a substantial part of iodine requirement in the human diet (Expert Group on Vitamins and Minerals, 2002) and iodine may enter the human diet through animal products, especially those of grazing animals (Slavin, 2005). Low dietary supply of iodine is considered the main factor for human iodine deficiency (Delange, 1994). Iodine content in the environment is considered to decrease with increasing distance from the sea (Whitehead, 1984). Therefore, people living in areas far removed from oceans require iodine supplements in their diet (Dunn, 1992). Strong soil iodine enrichment is limited to approximately 100-120 km from the coast and beyond this distance, iodine concentrations are close to its values found in central continental regions (Fuge, 2007). Crops grown in soils with low iodine content do not provide sufficient amount of iodine in the human diet (WHO, 2004).

Bioaccessibility of an element is a useful tool to assess the fraction of the total soil elemental content that can enter the human digestive system. There are no reports in literature on the assessment of bioaccessibility of any halogens from soil, although a study by Fukushima and Chatt (2012) estimated the bioaccessible iodine levels from Japanese seaweed and referred to the water-soluble iodine from the seaweed as the bioaccessible fraction. Bioaccessibility of an element from soil can be determined by the Physiologically Based Extraction Test (PBET) which simulates the leaching of soil in the human gastrointestinal tract, and determines the fraction of the total elemental content that is available for absorption (Ruby et al. 1993).

Endemic goitre was prevalent in the population of Derbyshire for many centuries until it declined from the 1930's possibly because of iodine supplements in foodstuffs, improved living standards, and increasing independency of people from their local environment for food (Saikat et al. 2004; Slavin, 2005). People living in the

carboniferous limestone areas of the Derbyshire Peak District were affected the most (Turton, 1933). The disease, colloquially known as the 'Derbyshire Neck', and the incidence of goitre in Derbyshire was higher in the carboniferous limestone area compared to other areas, particularly the surrounding millstone grit area (Turton, 1933). Yet, the iodine content in carboniferous limestone soils was higher than the millstone grit area, 5.5 mg/kg and 1.8 mg/kg, respectively (Turton, 1933), raising the question that the 'total' soil iodine content was not directly correlated to the incidence of goitre in these areas.

The reason why the Derbyshire limestone region was goitre-endemic has escaped a conclusive explanation (Orr 1931; Turton 1933; Fuge and Long 1989 and Saikat et al. 2004; Slavin, 2005). With regard to the oceans being the main provider of airborne iodine, no part of Derbyshire is more than 80 km distant from the sea, not considered remote compared to classical goitre areas such as the Alps (Fellenberg 1923) and the Himalayas (Kamarkar et al. 1974). When considering the direction of the prevailing wind (from the south-west), however, the area is 160 km away from the coast of Wales (Fuge and Long, 1989). Studies in the 1930's by Orr (1931) and Turton (1933) and more than fifty years later by Fuge and Long (1989), found no clear correlation between the iodine content in the environment and the incidence of goitre in the Derbyshire limestone region. It is to be noted that the pre-1930s studies were focussed mainly on a disease centred, medical viewpoint with not enough understanding of the mobility and interaction of iodine in the environment (Orr, 1931; Turton, 1933).

Environmental geochemical studies can help in assessing the quality of the environment as well as show the impact of the environment on human health and development of diseases (Varnavas et al. 2012). The aim of this study, therefore, is to establish an understanding of the causes of historic goitre in the UK-Peak District area. This is achieved through an assessment of soil bioavailabilty of iodine in the historic goitre-prevalent limestone area compared with the adjacent millstone grit areas of the UK- Peak District, followed by iodine human bioaccessibility studies (soil iodine bioaccessibility studies carried out here for the first time as noted from literature).

2. Experimental

2.1 *Study area and sampling strategies*

2.1.1 Study area

The study area, the UK-Peak District, is situated at the southern end of the Pennines in north Midland Britain. It lies mainly in the county of Derbyshire with some parts extending into the adjacent counties of Cheshire, Staffordshire and Yorkshire. Its geological structure comprises a central carboniferous limestone dome which is flanked successively by marine black shales, the millstone grit with alternate shales, and the sandstones and shales of the Lower Coal Measures (Ford, 1976).

2.1.2 Sampling Strategies

21 sampling areas were selected, 12 from the carboniferous limestone and 5 for comparison as background study areas from millstone grit region of the UK-Peak District. Sampling area selection was based on factors such as geology, accessibility, land ownership and land use for water, soil and availability of vegetation samples. In the limestone area, water samples were collected from a river (River Wye), ponds and lagoons, and in the millstone grit sites, from Rivers Derwent and Dove. Soil and vegetation samples were collected from agricultural or pasture land used for grazing. In order to minimise the effect of species variation, a perennial native plant (*Rumex* sp., commonly known as the Dock) abundant in majority of the study area was chosen as an example to estimate the iodine content of vegetation in the study area. Docks are said to be the most common perennial weed in grasslands, heaths, banks, cultivated ground and waste places in the UK (Clapham, 1969) and reproduce from seed and by vegetative regeneration from the underground organs. However, according to Karlsson (1952), Docks are not regarded as true weeds of grassland as they contribute to the herbage and are likely to contribute trace elements to the grazing animals' diet. Cattle, sheep, goats and deer graze on Docks (Bond and Turner, 2003) whilst introducing their mineral content into the food chain.

Samples were collected from three sampling sites from each sampling area. Composite sampling was carried out at each sampling site where samples were collected from three sampling points; at each sampling point, three sub-samples were collected, at 1m spacing of an arbitrary triangle, which were then bulked together, thus resulting in representative samples covering the study area (Keith, 1991 and 1996).

Water samples were collected in acid washed nalgene plastic bottles. They were filtered on-site through a 0.45μm Millipore filter paper fitted onto a plastic syringe. Soil samples (0-15 cm) were collected using a spiral hand auger and as soil adhering to the roots of *Rumex* sp where this plant was available, and placed in plastic bags. Samples of whole plants of *Rumex* sp of similar size and development stage were collected, the roots washed on site with distilled water, and the samples stored in polyethylene bags.

2.2. Sample preparation for water, soils and plants

The filtered water samples were acidified and stored below 4C until analysed.

Soil samples were air dried in Kraft bags (made of wet strength brown paper), disaggregated with a pestle and mortar as necessary, and then dry sieved to obtain the <2 mm particle size fraction. The <2 mm fractions were divided into two portions: one set was oven dried, tema milled (zirconium oxide mill) in agate pots to 100 μm grain size for 'total' iodine determination, and the other set was stored at room temperature for the pH, organic carbon, calcium content determination, and iodine bioavailability and human bioaccessibility experiments.

Plant samples were divided into different parts (roots, stems, leaves, seeds) in the laboratory and each part washed repeatedly with deionised water to remove any adhering soil. The samples were transferred into Kraft bags, air dried for two weeks and then oven dried at 40 C for 8 hours. Due to the fibrous nature of the *Rumex* sp., it

was not possible to mill it efficiently with a conventional plant mill. Instead, the plant samples were ground to a fine powder when frozen in liquid nitrogen and ground with a pestle and mortar (Brewin et al. 2007). Quality control measures employed included cleaning the pestle and mortar by rinsing with acetone, followed by three separate grindings of acid-washed sand. Milled plant parts were transferred to clean self-sealing polyethylene bags and stored in a desiccator at room temperature until analysed.

2.3. Analyses of samples

2.3.1 pH: The pH of water samples was measured on site using standard probes (Jenway 3051 pH meter). The pH of soil samples was measured in the laboratory using a soil:distilled water ratio of 1:2.5 (w:w) (Sakata, 1987) using a pH meter (Jenway 3051 pH meter).

2.3.2 Organic carbon and Calcium content of soils: The organic carbon content of soil samples was determined by the titration method (Gaudette et al. 1974). Soil samples were digested with nitric and perchloric acids (Thompson and Wood, 1982) and analysed for their calcium content by Atomic Absorption Spectrometry (AAS).

2.3.3 Iodine determination in water, soil, and plant samples:

2.3.3.1 Total iodine in water, soil, and plant samples

Iodine in the soil samples was extracted following the procedure of Rae and Malik (1996). Iodine from plant samples was extracted following the procedure of Watts *et al.* (2002) using TMAH (tetra methyl ammonium hydroxide). The extracts and water samples were analysed for their iodine content by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

2.3.3.2 Single and sequential extraction of iodine in soil samples

To assess the bioavailabilty and mobility of iodine from soils, single and sequential extractions were carried out on air dried soil samples.

Single extractions of soils were carried using water to assess readily available iodine, and acetic acid and hydrochloric acid to assess potentially mobilisable fractions. Extractions were carried out with cold distilled deionised water at room temperature (20 C) for 3 minutes (Johnson, 1980), hot water at 80 C for 3 minutes (Saikat, 2005), 0.42M acetic acid at 20 C for 16 hours (Whitehead 1973 a) and 0.01M hydrochloric acid at 37 C for 24 hours (Lottermoser, 2002). The extracts were stored below 4C and analysed by ICP-MS and the percentage of iodine extracted from the soils calculated (Johnson, 1980).

For the sequential extraction, soils with total iodine content closest to the mean iodine value (see Table 2) of the limestone and millstone grit study areas were selected. The method of arsenic fractionation of soil (Kavanagh et al. 1997) was followed because of its ability to indicate the association of an anion in soil as water-soluble, Al-associated, Fe and organic Fe-associated, Ca-associated, occluded Al-associated, and residual. The extracts were stored below 4 C until analysed by ICP-MS. Iodine concentration in the residual fraction was calculated by subtracting the sum of iodine concentrations in the five fractions from the 'total' iodine content of the sample. Iodine in each fraction was calculated as a percentage of the 'total' iodine concentration of the representative sample.

2.3.3.3 Human Bioaccessibility of iodine from soils using Phyiologically Based Extraction Test (PBET)

The PBET method has been developed as a routine-practical method for *in vitro* measurement of bioaccessibility of metals and metalloids (Wragg and Cave, 2002; Intawongse and Dean, 2007), and has shown to agree with *in vivo* studies, such as studies involving lead and arsenic in soils (Ruby et al. 1996; Rodriguez et al. 1999). There has been no report on the use of PBET for any of the halogens, including

iodine, however, the method can serve as a useful approximation of iodine bioaccessibility in humans.

The PBET method developed for arsenic by the British Geological Survey (Cave et al. 2002) was adapted to determine the bioaccessibility of iodine from soils in the present study. The test was carried out as a two stage sequential extraction using various enzymes to simulate both gastric and the small intestine compartments with extraction carried out at 37°C. Soil samples were introduced into the simulated gastric solution to solubilise any bioaccessible iodine present. The conditions were then modified after a stomach sample had been collected to simulate the small intestine. The PBET produced three results, the stomach phase bioaccessibility and two values for the intestine compartment (intestine I and II) representing 2 h and 4 h under the intestine compartment conditions. The extracts obtained to represent the stomach phase and the intestinal compartments bioaccesibility were analysed for iodine by ICP-MS. Iodine bioaccessibility in each sample was calculated as a percentage of the 'total' iodine concentration of the representative sample.

2.3.4 Quality control: Analysis of water, soil and plant samples for iodine was carried out in duplicate with regular running of 10 % reagent blanks and certified reference materials for 'total' iodine content in soils and plants. Iodine calibration solutions were run at regular intervals between the samples to check the consistency of results. Analysis of sample duplicates showed that the results were within ±10 %. Analysis of reagent blanks showed that there was no contamination or interference from the reagents. The certified reference materials (CRM) for soils included GBW soil 07401, GBW soil 07402, and GBW soil 07404 of the Institute of Geophysical and Geochemical Exploration, China, and for the vegetation was SRM 1573a (tomato leaves). The CRM percentage recovery for iodine was within ±10 % of the reference values.

2.3.5 Statistical Analysis: Microsoft Office Excel 2003 and SPSS were used to determine the descriptive statistics, Student's t-test and correlation coefficients.

'Transfer Factors' (Shinonaga et al. 2001) were determined as a ratio of the iodine concentration in the plant samples (mg/kg) and the iodine concentration in the respective soil sample (mg/kg) and as a ratio between the iodine content in the aerial plant parts and its roots to show iodine transport within the plant.

3. Results and Discussion

3.1 *Water pH and iodine content in the limestone and millstone grit sites*

Table 1 shows the means and ranges of pH and iodine content in the water samples in the study areas. pH of the water samples in both study areas are alkaline; pH in the limestone area is indicative of the underlying carboniferous geology of the Peak District (Alloway, 1995). The influence of limestone over the chemistry of water in the Peak District was also reported by Edmunds (1971) and with regards to the pH, hypothesised that limestone has a good buffering capacity by maintaining pH>7 which thus restricts the mobility of trace elements. pH of surface water in a millstone grit area of Ireland has been reported to range between 6.84-8.28 (Rothwell et al. 2005), which is agreement with the alkaline waters of the millstone grit area of the present study.

It is noted that the pH between the limestone and millstone grit areas is not significantly different (p=>0.05), however, iodine concentrations between the two sites are significantly different (p=0.027), with higher iodine in the surface water of the limestone area. This difference may not be a consequence of the site geology, but more a result of the samples collected - from still waters in the limestone area (mainly ponds, lagoons; only two river water sampling points) to running waters in the millstone grit sites (all samples from rivers) and therefore influenced by dilution.

The mean value for iodine in river water has been reported to be 5 μg/l (Fuge and Johnson, 1986), and in surface water of North England to be 3.71 μg/l (Fuge, 1989). In comparison with these values, iodine concentrations found in the limestone area (mean 4.8 μg/l) were comparable but levels found in the millstone grit area (mean 2.1

μg/l) were lower, although comparable if the upper end of the range of iodine is considered (Table 1).

Studies by McClendon and Williams (1923) showed that the rate of goitre is related to the iodine content of river water, with the goitre rate per 1000 men reported as 15-30 in river water containing 0-0.5 μg/l iodine, 5-15 in river water containing 0.5-2 μg/l iodine, and 0.1 in river water containing 3 and above 3 μg/l iodine. According to this data, the population in the millstone grit sites may be living in a relatively iodine deficient environment and therefore more prone to goitre than the limestone areas of the UK-Peak District, although literature suggests higher goitre rates in the limestone areas (Turton, 1933). Although iodine concentrations in water provide important information on its mobility in the environment (Fuge and Johnson, 1986), it is to be noted that with regards to endemic goitre, it is the bioavailability of iodine from the soil which is of more significance (Johnson, 2003; Stewart et al. 2003). The results presented here are indicative of the current status of iodine in the aquatic media of the study areas which can vary considerably over time periods. Hence the similarity of iodine content in water in the study areas in the current times should be considered only for general information, rather than definitive factors to determine the causes of historic goitre in the limestone area. Results obtained from the soil are therefore important determinants for the present study.

3.2 *pH, organic carbon, and iodine content of soil samples*

The means and ranges of the pH, organic carbon and iodine concentrations of soil samples of the limestone and millstone grit areas are given in Table 2. Mean pH values for soils in the limestone sites were near neutral and were acidic in the millstone grit sites. The percentage organic carbon was marginally higher in the millstone grit sites than in the limestone sites and this may be attributed to the difference in the extent of the vegetation cover between these study sites. In terms of a significant difference, soil Ca content was significantly higher ($p = 0.0003$) in the limestone areas than in the millstone grit areas and this is expected due to the

limestone geology of the area. It is noteworthy that the soil iodine concentration was not statistically different between the two study areas, indicating that the 'total' soil iodine may not necessarily be an important factor in determining the cause of goitre in the limestone area.

A number of world soil iodine values as world averages have been reported by different authors such as 5 mg/kg (Vinogradov, 1959), 1.2-5 mg/kg (Shaklette and Boerngen, 1984), 2.8 mg/kg (Kabata-Pendias and Pendias, 2001), and 3 mg/kg (Johnson, 2003); 1.27 mg/kg for a classical iodine deficient region in Missouri, USA (Fuge, 1987), and overall there is a broad range of reported soil concentrations from <0.1-150 mg/kg (Fuge, 2005). For the UK, mean soil iodine content of 11.2 and 9.2 mg/kg have been reported for England, Wales and Scotland respectively (Whitehead, 1973; Whitehead, 1979), 14.7 mg/kg for Wales (Fuge, 1987), 3 mg/kg for Derbyshire (Turton 1933), 5.4 mg/kg for Derbyshire limestone area (Fuge and Long, 1989). On comparing these values with those in the present study, iodine concentrations ranging from 0.74 – 12.14 mg/kg (mean 3.24 mg/kg) in the limestone area, and 0.56 - 4.65 mg/kg (mean 2.92 mg/kg) in the millstone grit soils (Table 2) indicate that the 'total' soil iodine content of the study area is comparable to the world averages, and higher than a classical iodine-deficient area. This again raises the question as to whether the 'total' soil iodine can be used to explain the reason for goitre in the limestone area of the present study.

Johnson (2003) has reported that it is difficult to consider any iodine concentration in soil as the typical value. Moreover, the concentrations in plants depend on the mobile and bioavailable iodine fractions in soil (Fuge and Johnson, 1986). Hence, with regards to human health, it is the bioaccessible/bioavailable fraction of the total soil iodine which is required before stating that an area is depleted with iodine (Johnson, 2003). Hence, it is not the 'total' soil iodine content which may be linked to goitre in the UK-Peak District historically, but a combination of several factors such as its mobility and bioavailability which may restrict its release into the food chain, and these aspects are explored further in this paper.

Soil pH, organic matter content, and concentrations of other major elements such as calcium, are important factors governing the 'total' iodine content of soil. In the limestone area, correlations between soil pH and 'total' iodine, and soil Ca and 'total' iodine were negative, and these were statistically significant (p= 0.00002; 0.022, respectively). In contrast, these correlations were positive although not statistically significant in the millstone grit area, suggesting that soil conditions and geology play an important role in the 'total' soil iodine content. Based on the pH correlation values, it appears that in the limestone area, an increase of pH corresponds to a decrease in iodine concentrations in the soil indicating that the mobility of iodine decreases with higher pH conditions. Reports on the relationship of soil iodine with pH in the literature are however, conflicting (Fuge, 1990). Some studies have found no relationship (Whitehead 1973), others have found fixation of iodine in high pH soils (Perel'man 1977), whereas some have reported that a high pH does not favour retention of iodine (Chilean Iodine Education Bureau 1956). It is noteworthy that the form of iodine which exists under given pH conditions may be important under alkaline conditions, free iodine present as I^{-1} is unlikely to be converted to the mobile form of iodine I_2, in turn, it is oxidised to the more stable IO_3^- (iodate) form, and hence is immobilised, as may be the case in the limestone area of the present study. In contrast, in acidic oxidising conditions, I^{-1} can be oxidised to I_2 (mobile or volatile form) (Fuge, 1996; Saikat et al. 2004) in the millstone grit area.

There is a significant positive correlation between organic carbon and iodine content of soils in the limestone area (p= 0.0007), thus indicating that organic matter is influential in restricting the mobility of iodine in this area (Fuge, 2005). A positive relationship between iodine and soil organic matter has been indicated by a number of earlier studies (Whitehead, 1973; Johnson, 1980; Sheppard and Thibault 1992; Kabata-Pendias and Pendias, 2001) although, Fuge and Long (1989) in their study on the Peak District soils did not find a marked correlation with organic matter content.

Therefore, with regards to endemic goitre, it is not the 'total' iodine in soil, but its mobility and bioavailability from the soil (Johnson, 2003; Stewart et al. 2003), as well

as human bioaccessibility, which are likely to provide an explanation as to why goitre was endemic in the limestone area historically and this is discussed below.

3.3. Iodine bioavailability and human bioaccessibility

3.3.1 Single extractions: Mean values of extracted iodine (as a % of 'total' soil iodine) in the limestone and millstone grit areas by the different extracting agents are shown in Table 3.

The cold water extraction represents the iodine fraction present as readily soluble salts and/or weakly adsorbed on the soil surface and is considered as mobile iodine (Johnson, 1980), whereas the hot water extraction is used to remove the simple polysaccharides and this extracts the iodine, indicating that it is water-soluble, and associated with the organic matter but not really complexed (Sheppard and Thibault, 1992). The % of iodine extracted by cold and hot water, and HCl were lower (although not statistically significant $p>0.05$) in the limestone area soils compared to the millstone grit soils indicating a lower solubility of iodine in the limestone area.

The highest percentage of iodine extracted by acetic acid may be related to the extractant's ability to release iodine from the more tightly bound exchangebale and Fe, Al oxides forms. Higher % iodine extraction by acetic acid for the limestone area soils as compared to the millstone grit area soils (Table 3) indicates that the iodine is more tightly bound in the limestone area soils, indicating lower mobility under normal conditions.

Correlation coefficients between the 'total' soil iodine and its % extracted using different extracting agents are shown in Table 4. Whereas a negative correlation of iodine extractability for all extracting agents with the 'total' iodine content from millstone grit soils was statistically significant, the relationship was not significant for the limestone area soils for cold/hot water extractions. Overall, a general trend of

negative correlation, similar to the findings of Johnson (1980), indicates that the content of ‘total’ soil iodine is not related to its increased mobility in the environment.

3.3.2 Sequential extractions: Sequential extraction of selected soil samples from the limestone and millstone grit areas were employed in order to estimate the distribution of iodine within the different operationally defined soil fractions. Partitioning of elements into the different soil fractions helps to enhance the understanding of its environmental mobility and availability for entering into the food chain (Mehra et al. 1999).

Iodine extracted in the different soil fractions as a percentage of the ‘total’ soil iodine is shown in Table 5. Similar to the cold water single extraction findings (Table 3), the percentage of iodine extracted as water-soluble by sequential extraction is low (mean 5.43% in the limestone area, 2.5% in the millstone grit area (Table 5). Overall, the low iodine extractability in the readily soluble fraction (water-soluble) as shown in the single (Table 3) and sequential (Table 5) extraction experiments and the high percentage of iodine in the unavailable residual soil fraction (Table 5) indicate that a high percentage of the iodine in the study area is tightly bound in the soil.

The order of iodine extraction into the different soil fractions is as follows:
limestone area: Fe & org Fe-associated > residual >> Al-assoc > Ca-assoc > water-soluble > occluded Al-assoc
millstone grit area: Fe & org Fe-associated > residual >> Al-assoc > water-soluble > occluded Al-assoc > Ca-assoc

Much higher iodine association with calcium in the limestone area (10.2%) compared with the millstone grit area soils (no values obtained – below detection limit of analysis) (Table 5) again indicates that the iodine is strongly bound with calcium in the soil matrix of the limestone area, and hence does not exist in a freely available form in this area.

3.3.3 Iodine content in *Rumex* sp.

Kabata-Pendias and Pendias (2001) have pointed out that for an effective evaluation of the pool of bioavailable trace elements from the environment, techniques based on both soil tests and plant analyses should be used together. Moreover, the uptake of trace elements by different plants from the same soil environment can be quite variable (Kabata-Pendias and Pendias (2001). As pointed out in section 2.1.2, *Rumex* sp. were selected for the present study due to its availability across all study sites, and also due to their likely contribution of trace elements to the grazing animals' diet as cattle, sheep, goats and deer graze on these plants (Bond and Turner, 2003) whilst introducing their mineral content into the food chain.

Iodine distribution in different parts of the *Rumex* sp. and the soil in which these plants were growing is shown as mean values in Table 6. The overall iodine content in *Rumex sp*. in the limestone and millstone grit sites followed the order:

Limestone area: roots>leaves>seeds>stems

Millstone grit area: leaves>seeds>roots>stems

This is in agreement with studies by Weng et al. (2008) who have reported that the absorbed iodine in a plant is not uniformly distributed among plant tissues. According to Whitehead (1973), Zhu et al. (2003), Weng et al. (2008), iodine taken up from soil is mainly retained in the roots and transport to the shoots may increase with an increase of soil iodine. These findings agree only to some extent with the present study, where it can be seen that although iodine is mainly retained in the roots, this is so only in the limestone area plants; moreover, transport to the shoots does not show an increase with an increase of soil iodine (Table 6).

Iodine uptake in the roots in the limestone area was significantly higher than in the millstone grit area (Table 6, $p=0.005$), however, there was no significant difference in the iodine content of the aerial plant parts between the two study areas. Transfer factor (TF) values of iodine in *Rumex* sp. roots *vs* soil, aerial plant parts *vs* soil, and

aerial plant parts *vs* roots are shown in Figure 1 which shows that the ratio of iodine uptake from the soil was higher in the roots than in the aerial plant parts in the limestone area, but this was not the case in the millstone grit area. The TFs also showed that the ratio of iodine in aerial plant parts as compared to their soil iodine content were similar in the limestone and millstone grit study sites (TF=0.30, 0.33 in the limestone, millstone grit areas, respectively). However, it is particularly noteworthy that the iodine taken up in the roots was readily transported to the aerial plant parts in the millstone grit study plants (TF = 1.42) unlike the limestone sites (TF = 0.49) showing that the iodine taken up by the roots flows freely to the aerial plant parts and hence into the food chain in the millstone grit sites, but is restricted in the roots in the limestone area. As cattle graze on the aerial plant parts, and where aerial plant parts are used for human consumption, these findings lead to suggest that the transfer of iodine into the food chain (directly through plants and indirectly in the diet through milk or meat from grazing cattle) in the limestone area is considerably lower than in the millstone grit area.

Iodine content in grass and herbage from different countries has been shown to be around 0.2 mg/kg; and for the UK it has an estimated mean value of 0.22 mg/kg (Whitehead, 1984). It is noted that the iodine content of aerial plant parts in present study area (Table 6) are comparable to the reported concentrations, thus indicating a low uptake into the plants which is in agreement with Fuge (2005) and Slavin (2005) who have stated that the iodine content of plants is generally low.

The study of Orr (1931) measured iodine concentrations in pasture samples in relation to the goitre problem in England and Scotland and reported values of 0.66 and 0.57 mg/kg in the high and low goitre incidence areas, respectively. These values are comparable to the iodine concentrations in aerial plant parts of *Rumex* sp in the present study (0.46 and 0.43 mg/kg in the limestone and millstone grit study sites, respectively, Table 6).

3.3.4 Iodine human bioaccessibility

The percentage bioaccessible iodine in the different phases of the gastrointestinal tract as simulated by the PBET in the limestone and millstone grit area soils is shown in Table 7. The results show that although the soil iodine bioaccessibility in the stomach phase was comparable between the two study areas, the millstone grit soils showed higher iodine bioaccessibility in the intestinal phases, thus providing a higher concentration of iodine into the human system in the millstone grit area (however, these differences are not statistically significant). The lower iodine bioaccessibility in the intestinal phases in the limestone area could be linked to its lower solubility and bioavailability in the calcium rich soils.

Correlations of bioaccessible iodine and soil pH were negative for both study areas, but the relationships were not significant. Soil Ca content showed a significant negative correlation with the iodine bioaccessibility in the intestine phase I for the limestone soils (P=0.04); all other correlations were negative, but not significant. These results again indicate that calcium has played an important role in rendering the iodine bio-unaccessible in the limestone area.

The experiment and findings on iodine bioaccesibility using the PBET are the first of their kind in the literature; and there is an absence of any literature on iodine or other halogens bioaccessibility studies. These findings therefore provide a basis for future studies in this field.

4. Conclusions:

The findings of this study show that:

- Soil pH, organic matter, and calcium content influence the 'total' iodine content and its mobility in the study area soils. Higher pH and calcium content of the limestone area as compared with the millstone grit area soils are influential in restricting the mobility of iodine in the limestone area; moreover, a significant positive correlation between soil organic carbon and iodine

content in the limestone area indicates that organic matter is influential in fixing the iodine in the soil in this area.

- The ‘total’ soil iodine content of the goitre-endemic limestone area and the background millstone grit area are not statistically different, also these concentrations are comparable to world background literature values, suggesting that the ‘total’ environmental iodine may not be linked to goitre in the UK-Peak District limestone area. However, soil iodine bioavailability and bioaccessibility in these areas are likely to provide an answer to the study aim.
- Lower % iodine (expressed as a % of the total soil iodine) extracted by cold water, hot water, and HCl for the limestone area soils as compared with the millstone grit soils indicates lower iodine bioavailability and mobility in the goitre-endemic limestone area.
- Higher % iodine (expressed as a % of the total soil iodine) extracted by acetic acid for the limestone area soils as compared with the millstone grit soils indicates that the iodine is more tightly bound in these soils, and hence not readily bioavailable.
- Sequential extraction studies show a much higher association of iodine with calcium in the limestone area than in the millstone grit area, indicating that the iodine is strongly bound with calcium in the soil matrix of the limestone area, reducing its bioavailability in this environment.
- Transfer factors of iodine as a ratio of iodine in the aerial plant parts *vs*. the roots and soil indicates that the transfer of iodine into the food chain in the limestone area is restricted and is considerably lower than in the millstone grit area. Iodine taken up by roots is transported freely into the aerial plant parts in the millstone grit area as compared with the limestone area, thus providing higher iodine into the human food chain through grazing animals in the millstone grit area.
- Higher iodine bioaccessibility in the millstone grit area than in the goitre-endemic limestone area suggests that oral bioaccessibility is a contributory factor to a higher intake of iodine into the human system in the millstone grit area and a lower intake of iodine in the limestone area. A significant negative correlation between soil calcium content and iodine bioaccessibility in the

limestone area indicates that calcium has played an important role in tightly binding the iodine to soils in this area, hence reducing its bioaccessibility.

- These findings suggest that the causes of goitre in the limestone area of the UK-Peak District area are linked to the low iodine mobility, bioavailability, bioaccessibility and transfer into the food chain in this area as people in those times depended on locally grown food for their daily dietary intake. However, in the present times, with the advancement of science and agriculture, and provision of dietary supplements, it is possible to have a balanced diet through locally grown food.

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Table 1 Water pH and iodine content in the study sites

Study Sites	**pH**	**Iodine (μg/L)**
Limestone area (n=36)		
Mean	8.8	4.84

Range SD	8.2-10.6 0.7	1.6-10.8 3.59
Millstone Grit area (n=15) Mean Range SD	 8.6 8.2-9.0 0.4	 2.10 1.70-3.70 0.89

SD = standard deviation

Table 2 Soil pH, organic carbon and iodine content in the study sites

Study sites	**pH**	**Organic carbon (%)**	**Ca g/kg**	**Iodine mg/kg**
Limestone area (n=36) Mean	 6.6 4.6-7.6	 2.72 0.51-7.93	 81.40 0.79-162.50	 3.24 0.74-12.14

Range SD	0.74	2.19	57.61	3.06
Millstone Grit **area** (n=15) Mean Range SD	 5.5 3.5-6.4 1.08	 3.49 0.57-6.35 2.45	 12.28 2.62-31.02 12.41	 2.92 0.56-4.65 1.34

SD = standard deviation

Table 3 Single extraction of soil iodine treated with cold water, hot water, 0.01 M HCl, 0.42 M CH3COOH (expressed as % of total iodine)

Study site	**Cold water**	**Hot water**	**HCl**	**CH_3COOH**
Limestone area (n=36) Mean Range SD	 2.65 BDL-14.8 4.72	 16.43 BDL-56.52 14.13	 7.86 1.3-17.9 6.65	 34.87 5.0-86 27.75
Millstone Grit area				

(n=15) Mean Range SD	6.49 BDL-32.14 12.73	17.18 3.22-42.85 18.12	8.17 1.2-20.7 7.19	23.05 2.6-45 16.82

SD = standard deviation; BDL = below detection limit of the analytical technique

Table 4. Correlation coefficients between 'total' and percentage extracted iodine from soil from the study sites (p value in brackets)

Study sites	**Cold water**	**Hot water**	**HCl**	**CH_3COOH**
Limestone area (n=36)	-0.038	-0.29	-0.51 (0.051**)	-0.58 (0.023**)
Millstone Grit area (n=15)	-0.87 (0.02**)	-0.84 (0.037**)	-0.95 (0.003**)	-0.87 (0.024**)

**Correlation is significant at the 0.05 level (2-tailed); correlation not significant (P>0.05)

Table 5. Sequential extraction of soil iodine (expressed as % of total iodine)

Study sites	**'Total' soil Iodine (mg/kg)**	**Water-soluble**	**Al-assoc**	**Fe and org Fe-assoc**	**Ca-assoc**	**Al- assoc (Occluded)**	**Residual**
Limestone area (n=15) Mean	3.16	5.43	14.9	34.67	10.24	1.91	32.85

Range SD	1.84-6.6 1.97	2-10.3 4.33	11.5-21.2 5.46	16.5-48.5 16.43	1.52-15 7.56	BDL-5.6 3.19	18.9-55 19.40
Millstone grit area (n=6) Mean Range SD	3.25 3.14-3.36 0.16	2.5 1.5-3.5 1.41	12.45 9-15.9 4.88	43.3 28-58.6 21.64	BDL BDL	0.96 BDL-1.91 1.35	40.80 20.09-61.5 29.28

SD = standard deviation; BDL = below detection limit of the analytical technique

Table 6. Iodine uptake by *Rumex* sp. (mg/kg)

Study sites	**Soil Iodine**	**Roots**	Stems (S)	Leaves (L)	Seeds (Se)	**Aerial (S+L+Se)**
Limestone area (n=21) Mean Range SD	2.46 0.75-6.5 1.92	1.05 0.68-1.88 0.39	0.25 0.11-0.48 0.11	0.71 0.16-1.3 0.44	0.41 0.27-0.58 0.12	0.46 0.24-0.73 0.18

Millstone grit area (n=9)						
Mean	2.87	0.37	0.29	0.55	0.46	0.43
Range	0.6-4.7	0.17-0.52	0.20-0.42	0.41-0.73	0.45-0.47	0.40-0.46
SD	2.08	0.18	0.11	0.16	0.01	0.03

SD = standard deviation

Table 7. Iodine bioaccessibility in the different phases of the gastrointestinal tract (expressed as % of 'total' iodine)

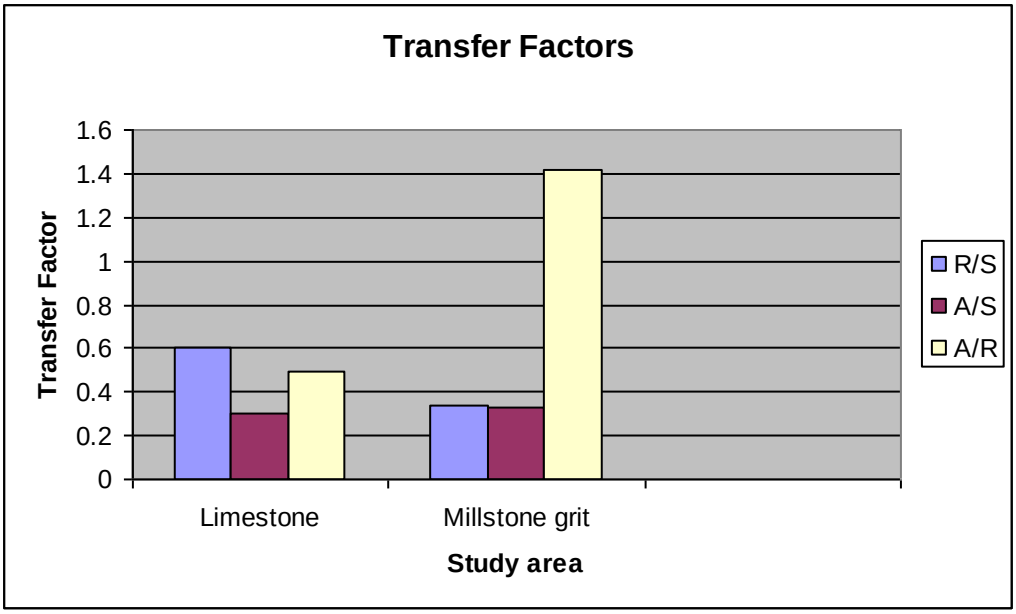
Study site	**Stomach phase**	**Intestine Phase I**	**Intestine Phase II**
Limestone area (n=21)			
Mean	16.4	13.4	13.1
Range	10-20.6	6-21	5.5-23
SD	2.0	1.8	2.2

Millstone Grit area (n=9)			
Mean	17	20.6	18.6
Range	7-27	11-27	10-32
SD	5.7	7.3	6.7

SD = standard deviation

Figure caption:

Figure 1 Transfer factor (TF) values for iodine in *Rumex* sp. in the limestone and millstone grit areas of the UK-Peak District


Transfer Factors
Transfer Factor
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Limestone
Millstone grit
Study area
R/S
A/S
A/R